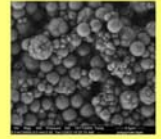
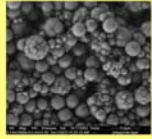


# Point-of-Use potentiometric immunosensor for environmental screening and food quality assurance

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## Abstract

Inexpensive polypyrrole coated screen-printed carbon electrodes with an integrated reference electrode are combined with established competitive ELISA techniques, to produce rapid (<15mins), sensitive (<50ppt) and reproducible biosensors which work in complex fluids such as milk, homogenates, serum, blood and urine. The sensors are monitored at point-of-use using a handheld potentiostat or a Pocket PC.

Bound antibody-target-label complexes formed at the surface of the immunosensor are detected potentiometrically, using a "charge-step" procedure. A shift in potential is measured at the sensor surface due to local changes in redox state and pH caused by the activity of the horseradish-peroxidase (HRP) label.

This ultrasensitive potentiometric technology has previously been demonstrated in clinical assays for hepatitis B surface antigen, Troponin I, and Digoxin, but is now being applied to the more demanding sectors of environmental testing and food quality assurance.

Handheld instruments and assays are being developed initially for detection of antibiotics and mycotoxins in milk as well as for soil contaminants such as PCB

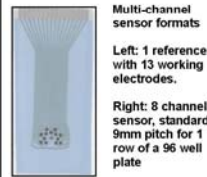
## Typical results after initial assay transfer

Diagnostic Use	Target	Molecular Weight	Dynamic Range	Sensitivity
Diseases	HbsAg	300 -1000 Da	<0.05ng/ml - 10µg/ml	50fM
	Troponin I	23 Da	<0.05ng/ml - 100ng/ml	~2pM
Markers	hCG	36.7kDa	0.5ng/ml - 100ng/ml	~13pM
	hTNF	21-28kDa	<0.008ng/ml - 1ng/ml	~0.3pM
Drugs	Digoxin	780 Da	0.5ng/ml - >2ng/ml	Therapeutic window
Toxins	Aflatoxin M1	328 Da	< 250ppt - >2000ppt	< 0.7nM
Contaminants	Penicillin G	372.5 Da	<1ppb - >10ppb	~2.7nM
	Chloramphenicol	323 Da	<50ppt - >1.2ppb	~0.15nM

## Instrumentation and Sensors



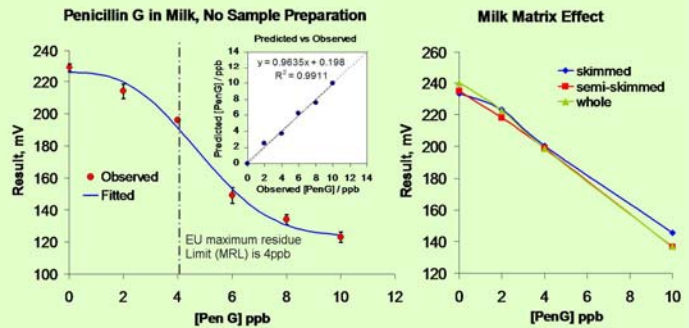
Various sensor formats (actual size) with gold or carbon working electrodes and Ag/AgCl reference electrodes



Other electrochemical sensors could be integrated: amperometric, impedance, capacitance etc

## Example: Penicillin G Assay

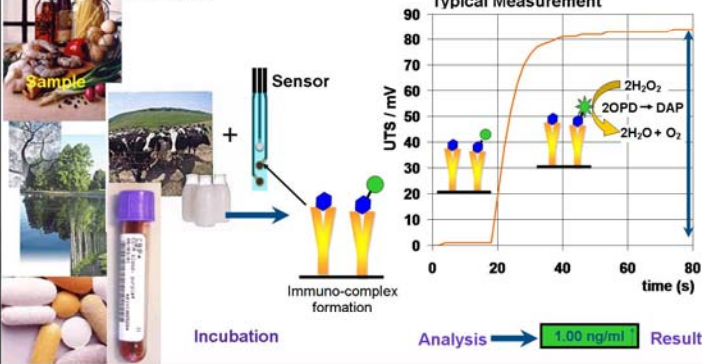
A 1 ml milk sample was incubated for 10 minutes in a vial containing 25ul of a stabilised solution of a penicillin-binding-protein-/horseradish-peroxidase complex (R39-/HRP). A β-lactam-conjugate coated biosensor was then applied to the sample mixture and incubated for a further 2 minutes. The biosensors were then rinsed and measured. The total time to perform this test is 15 minutes. The results can be seen below.



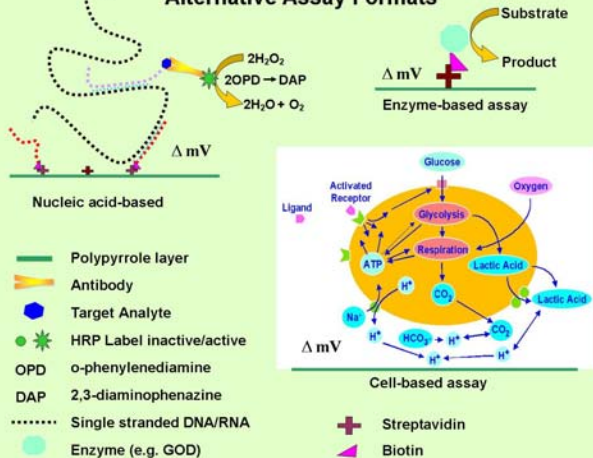
A standard five parameter log-logistic model was used to curve fit the data. Each point represents 40 samples, measured over a period of 8 days, using 5 different sensor batches. Error bars = ± 1 x standard deviation. In addition, experiments were performed using milk with different fat content to gauge the matrix effect, the results of which are also illustrated above. Similar results are obtained at RT (~18-24°C)

Future systems based on semi-automated processes will take 5 - 10 minutes depending on required sensitivity. Fully automated systems based on active cartridge fluidics will take ~2 minutes.

## Process



## Alternative Assay Formats



## Validated Results

Blind Sample Testing: No Sample Preparation  
Independently supplied reconstituted freeze dried milk samples

Sample	Penicillin G (ng/ml, ppb)		Chloramphenicol (ng/ml, ppb)	
	Given	Measured	Given	Measured
1	0	0	0	0.05
2	2	2	0	0
3	4	4	0	0
4	6	5	0	0
5	8	7	0	0
6	10	8	0	0
7	0	0	0	0
8	0	0	0.05	0.05
9	0	0	0.1	0.05
10	0	0	0.2	0.1
11	0	0	0.3	0.2
12	0	0	0.5	0.4
13	0	0	1.0	1.0
14	0	0	0	0
15	4	4	0.3	0.2
16	8	7	0.5	0.3

## USP

- Precise quantitative measurements in complex sample matrices
- Little or no sample preparation
- Rapid and simple testing regime
- Well established assays can be directly transferred to the technology
- Universal and flexible (proteins, nucleic acids, toxins, drugs, contaminants and cells)
- Accurate and reproducible
- Long term stability
- Low cost and suitable for automation

## Acknowledgements:

We would like to thank Dr Jacques Degelaen (UCB-Bioproducts) for supplying the reagents and expertise during the development of the Penicillin G assay illustrated here.